



# Phytochemical content and analgesic activity of ethanol extract of *Hibiscus Sabdariffa* in experimental animal model

Omodamiro OD<sup>1</sup>, Achi NK<sup>1</sup>, Jimoh MA<sup>2</sup>

1. Pharmacology unit, Department of Biochemistry, College of Natural Sciences, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria

2. Department of plant science and Biotechnology, College of Natural Sciences, Micheal Okpara University of Agriculture, Umudike, Nigeria

## ✉Corresponding Author:

Dr. Omodamiro O.D,

Email: majekdamiro@yahoo.com

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## General Note



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## ABSTRACT

This research evaluated the phytochemical contents and analgesic properties of ethanol extract of *Hibiscus sabdariffa*. Fresh leaves of *H. Sabdariffa* were obtained, dried and milled. The samples were then analyzed for phytochemical composition using standard methods. The analgesic property was done using 20 wistar albino rats weighing 80-120 g and grouped into 5 different groups (n=4). Group one served as control and received only feed and water *ad libitum*, group 2: received morphine, intraperitoneally and Group 3, 4, and 5 received *Hibiscus sabdariffa* ethanol leaf extract (250, 500, 750 mg/kg) orally, respectively. The photochemical result

shows the presence of flavonoids (28.66 %) and tannins (30.43 %) as the highest phytochemicals while others are alkaloids (20.74 %), phenols (18.93 %), cardiac glycosides (5.70 %) and saponins (15.60 %). The analgesic result showed that significance percentage inhibition of writhing ( $p < 0.05$ ) at 750 mg/kg bw which showed similar potency as morphine. Therefore, these results show that *H. Sabdariffa* can be used as an analgesic when consumed in a high concentration.

**Key words:** Phytochemical, analgesic, *Hibiscus Sabdariffa*

## 1. INTRODUCTION

Plants are composed entirely of chemicals of various kinds (Molyneux *et al.*, 2007). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 2009). The phytochemical category includes compounds recognized as essential nutrients, which are naturally contained in plants and are required for normal physiological functions, so must be obtained from the diet in humans (Timbrell, 2005). This phytochemicals found in plant are the major source of medicinal potency of plants.

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone (Adam, 2010). All of these drugs present well known side and toxic effects. On the contrary many medicines of plant origin had been used since long time without much adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs.

*Hibiscus sabdariffa* is an annual or perennial herb or woody-based sub shrub, growing to 2–2.5 m tall. It is distributed in the Indian subcontinent, Bangladesh, Myanmar, Thailand, Senegal, Mali, Niger, Congo, France, Gambia, Nigeria, Egypt, Sudan, Namibia, Caribbean Panama, Indonesia and Malaysia. The plant is considered to have antihypertensive properties. In East Africa, the calyx infusion, called "Sudan tea", is taken to relieve coughs. The heated leaves are applied to cracks in the feet and on boils and ulcers to speed maturation (Aune *et al.*, 2012).



**Figure 1** *Hibiscus sabdariffa* L., (Obouayeba *et al.*, 2014).

A lotion made from leaves is used on sores and wounds. The seeds are said to be diuretic and tonic in action and the brownish-yellow seed oil is claimed to heal sores on camels. In India, a decoction of the seeds is given to relieve dysuria, and mild cases of dyspepsia. Brazilians attribute stomachic, emollient and resolutive properties to the bitter roots (Obouayeba *et al.*, 2014). Because of all the medicinal claims of *Hibiscus sabdariffa*, this study was designed to assess the phytochemical content and analgesic activity of ethanol extract of *Hibiscus sabdariffa* in experimental animal model.

## 2. MATERIALS AND METHODS

### Sample Collection/Identification

*Hibiscus sabdariffa* leaves were purchased from Urban Main Market Umuahia, Abia State Nigeria. The plant was identified and authenticated by Dr. Blessing Mbaebie Oyedemi of Department of Plant Science and Biotechnology, Michael Okpara University of

Agriculture Umuduke Abia State, Nigeria. The leaves were air dried for 48hrs, then ground to powder and stored in an air tight container.

### Preparation of Extracts

The extraction was done using maceration method. About 100g of dried ground *Hibiscus sabdariffa* leave sample was weighed and macerated in 500ml of ethanol. The solution was left for 72 hours to ensure proper extraction. The solution was then filtered using a filter paper, the filtrate obtained was concentrated by evaporation using water bath (45°C) and then stored at room temperature for further use.

### Experimental Animals

All animals were handled according to method described by guide for care and use of laboratory animals (1996). Adult wistar albino rats weighing 130-160g were obtained from the laboratory animal unit of the Department of veterinary physiology, Animal health and production, University of Nigeria Nsukka. The animals were housed in standard husbandry condition for acclimatization periods of 21 days and fed with commercial pellet diet (vital growers mash by grand cereal and oil mills, Nigeria) before the experiment was performed.

### Acute Toxicity Study

Twenty male mice were used for this experiment. They are randomly divided into five groups of four animals each and housed in separate cages. The rats in groups 1-5 were treated orally with 200, 400, 600, 800, 1000mg/kg, respectively of *Hibiscus sabdariffa* extract. The rats were allowed access to feed and water ad libitum and were observed for 48 hours for signs of toxicity and mortality (Madubunyi et al., 2012).

### Quantitative Phytochemical analyses of Hibiscus sabdariffa leave

The amount of each phytochemical: flavonoids, phenols, alkaloids, saponins, tannins, Cyanogenic Glycoside present in *Hibiscus sabdariffa* powdered sample were evaluated using standard laboratory procedures based on the methods of Chang et al., (2002), Singleton et al., (1999), Harborne (1973), Van-Burden and Robinson (1981), Amadi et al. (2004).

### Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al., 2002). 1 mL of *Hibiscus sabdariffa* plant extracts (1 mg/mL) is mixed with 1 mL of AlCl<sub>3</sub> 2%, after 10 minutes of incubation, the absorbance were measured at 415 nm. Quercetin was used as a standard. Results were expressed as mg of quercetin equivalents (EQ) per 100 g of extract.

### Determination of total phenol content

The total phenol content of the *Hibiscus sabdariffa* extracts was determined using the method reported by Singleton et al., (1999). Appropriate dilutions of the extracts (0.5ml) was oxidized with 2.5ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard.

### Determination of alkaloids using Harborne (1973) method

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The solution was then filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue if present is the alkaloid which is dried and weighed.

### Determination of Saponin

0.5 g of the *Hibiscus sabdariffa* plant extract was added to 20 ml of 1N HCl and boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5ml of acetone ethanol was added to the residue and 0.4mls of each taken into 3 different test tubes. Ferrous sulphate reagent (6ml) was then added each followed by 2 ml of conc. H<sub>2</sub>SO<sub>4</sub>. This was thoroughly mixed after 10 min and the absorbance taken at 490 nm (Oloyed, 2005). The absorbance of saponin standard solution was read after color development at same wavelength of 490nm.

### Determination of tannin by the method of Van-Burden and Robinson (1981):

500 mg (0.5g) of the plant sample was weighed into a 50 ml beaker. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1M FeCl<sub>3</sub> in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

### Determination of Cyanogenic Glycoside

Cyanogenic glycoside was quantitatively determined using the method of Amadi et al. (2004) as reported by Ejikeme et al. (2014). One gram of dry *Hibiscus sabdariffa* powder was weighed into a 250cm<sup>3</sup> round bottom flask and 200cm<sup>3</sup> of distilled water was added and allowed to stand for 2 hours for autolysis to occur. An antifoaming agent (tannic acid) was added and full distillation carried out in a 250cm<sup>3</sup> conical flask containing 20cm<sup>3</sup> of 2.5%NaOH (sodium hydroxide). To 100cm<sup>3</sup> of each distillate containing cyanogenic glycoside, 8cm<sup>3</sup> of 6M NH<sub>4</sub>OH (ammonium hydroxide) and 2cm<sup>3</sup> of 5% KI (Potassium Iodide) was added, mixed and titrated with 0.02M AgNO<sub>3</sub> (silver nitrate) using a micro-burette against a black background. Permanent turbidity indicates the end point (Amadiet al., 2004).

Cyanogenic glycoside content of the sample was calculated as:

$$\text{Cyanogenic glycoside } \frac{\left( \text{mg} \right)}{100\text{g}} = \frac{\text{Titre Value (Cm}^3\text{)} \times 1.08 \times \text{exact volume}}{\text{Aliquot volume (Cm}^3\text{)} \times \text{sample weight (g)}} \times 100$$

### Qualitative determination of phytochemical contents of *Hibiscus sabdariffa*

*Hibiscus sabdariffa* plant extract were used for these screening using standard procedures described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

## ANALGESIC STUDY

### Tail Flick Response in rats

Twenty (20) albino rats were used for this experiment. They were randomly divided into five groups of four animals in each cage labeled group 1,2,3,4and 5, respectively, with group 1(control group) received normal saline, Group 2 (standard) received morphine, intraperitoneally and Group 3, 4, and 5 received *Hibiscus sabdariffa* leave extract (250, 500, 750mg/kg) orally. The animals were fasted for 24hours but given adequate amount of water. About 5cm of the rats tail was immersed into a hot water maintained at 55±1°C and the tail withdrawal reflex period (latency) was taken at 0, 15, 30, 60, 90 minutes, (I.e; the time taken for the rat to flick its tail, known as Pain Reaction Time (PRT). was recorded for all the rats.) after oral administration of extracts drugs and normal saline (Adzu et al., 2001).

### Acetic Acid Induced Avdominal Writhing in Albino Rats

Twenty (20) albino rats were used for this study. They were randomly divided into five groups of four animals in each cage labelled group 1, 2, 3, 4 and 5 respectively. The animals where fasted for 24hours but given adequate water. Writhing was induced in the rats according to a method described by Vale et al., (2003). The twenty four hours fasted rats were injected intra peritoneally with 0.2ml of 3% acetic acid solution one hour after oral administration of the extracts, morphine and normal saline. The number of writhing was observed between 5 and 15mins. A reduction in the number of writhing was compared with the control group and was considered as evidence for analgesia. Index of Analgesia was expressed as percentage inhibition of writhing and calculated according to the following formulae:

$$\% \text{ inhibition} = (\text{MWc} - \text{MWt}) \times 100 / \text{MWc}$$

Where MWc=Mean number of writhing in the control

MWt= Mean number of writhing in treated group.

### 3. RESULTS

**Table 1** Qualitative Analysis of *Hibiscus sabdariffa*

Phytochemical	Quality
Flavonoid	+++
Alkaloid	++
Phenol	++
Cardiac glycoside	+
Tannins	+++
Saponins	++

+ = **moderately present**

++ = **present**

+++ = **very present**

**Table 2** Quantitative analysis of *Hibiscus sabdariffa*

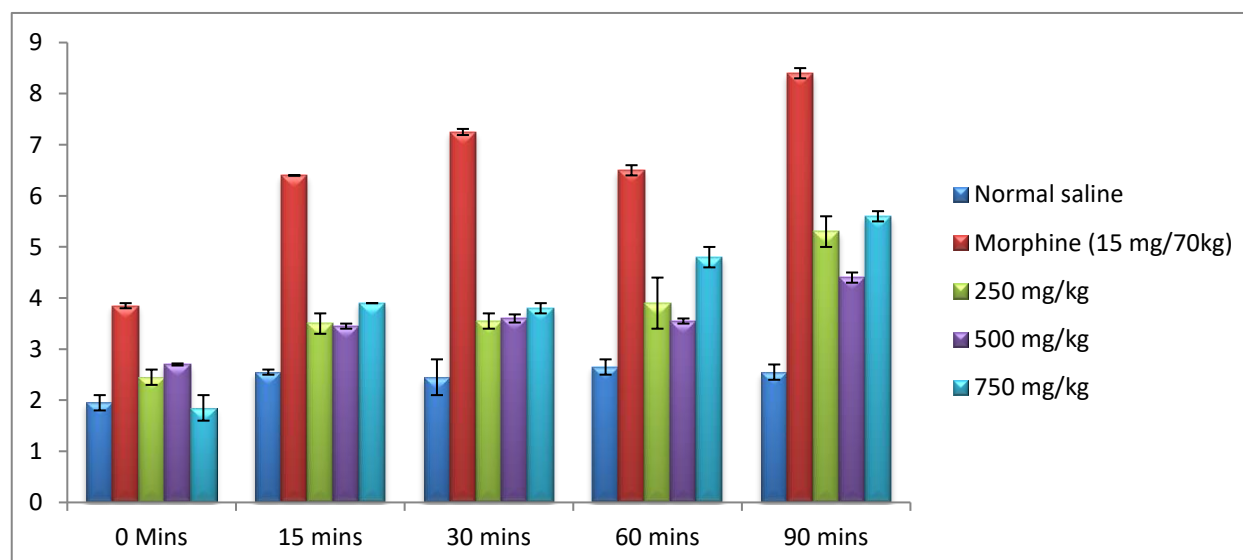
Phytochemical	%
Flavonoid	28.66
Alkaloid	20.74
Phenol	18.93
Cardiac glycosides	5.70
Tannins	30.43
Saponins	15.60

**Table 3** Inhibition rate of *Hibiscus sabdariffa* on wistar albino rats

Normal saline	<b>9.00 ± 1.00<sup>a</sup></b>	-
Morphine (15 mg/70kg)	4.50 ± 0.50 <sup>b</sup>	50.0
250 mg/kg	5.00 ± 0.00 <sup>b</sup>	44.4
500 mg/kg	4.50 ± 0.50 <sup>b</sup>	50.0
750 mg/kg	2.00 ± 0.00 <sup>c</sup>	55.6

The results with different superscript are significantly ( $p < 0.05$ ) different when compared with control and between groups.

#### Analgesic effect of *Hibiscus sabdariffa* on wistar albino rats



**Figure 2** The figure above shows that the extract had a dose-dependent increase in analgesic properties. The result also shows that the standard control (morphine) had a significant ( $p < 0.05$ ) increase in analgesic property when compared with the extract

## 4. DISCUSSION

The result of qualitative analysis of *H. sabdariffa* shows that flavonoids and tannins are the most identified. This was followed closely by alkaloids, phenols and saponins while cardiac glycosides were the least identified. This is in agreement with the results of Adegunloye *et al.*, (1996) and Okereke *et al.*, (2015). The economic uses of the plant are not limited in making drink and as medicinal plant (Onyenekwe *et al.*, 1999). In Africa, the water extract of *Hibiscus sabdariffa* is taken as hot or cold drink. The leaves and calyces are used as vegetables in various local dishes (Obiefuna *et al.*, 1996). *Hibiscus sabdariffa* plant is antiseptic, diuretic, purgative, sedative and emollient. The leaves in combination with ginger are used to suppress high blood pressure and in treatment of hypertension (Haji and, Haji, 1999). Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agent (kiyama *et al.*, 2001). Tannins have been demonstrated antibacterial activities. With proline-rich proteins, tannins form irreversible complexes which may be able to inhibit the cell-wall-protein synthesis of bacteria (Hagerman and Butler, 1981). Some alkaloids from plants have also been used as antimicrobials in food (Hintz *et al.*, 2015). Recently, saponins have been used as a preservative and/or used as a part of a preservative system to inhibit and/or reduce growth of spoilage microorganisms of beverages and foods.

Acetic acid induced writhing test is a model of visceral pain which is a very sensitive test for analgesic drugs (Vyklícký, 2000). In the acetic acid induced writhing models, results show that the extracts significantly reduced writhing response. As intraperitoneal injection of the acid produced abdominal writhing by activating chemo-sensitive nociceptors but the extracts were able to protect the animals, thus exhibiting analgesia. At the high dose of 750mg/kg, there was similar analgesic effect to that of the standard drug morphine. The dose dependent inhibition of acetic acid induced writhing by the extracts indicated a peripheral effect and it's suggestive of the dose dependent manner of medicinal plants extracts in the treatment of pain and inflammation (Olajide *et al.*, 2000). The inhibition of acetic acid writhing shows that the extracts may have central effects on the nervous system and depressant effect on the nervous system since central nervous system depressants have been known to inhibit or reduce the number of writhing in acetic acid pain models (Stevenson *et al.*, 2009). The outcome of the tail flick test showed that the extracts inhibited the thermally induced nociceptive spinal reflex in the rats in a dose and time dependent manner with the maximum analgesic effect being reached at 90 minutes  $p < 0.05$ . The analgesic effect of the extracts is closely related, and similar to that seen in morphine, and may be due to the presence of alkaloids in the extract which acts through opioid receptors (Farouk *et al.*, 2008).

## 5. CONCLUSION

The results from this research prove that *H. Sabdariffa* is a good source of active phytochemicals and also possess some analgesic properties. This supports the use of *H. Sabdariffa* as a medicinal plant for the treatment of many diseases.

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**Conflict of Interest:** The authors declare that there are no conflicts of interests.

**Data and materials availability:** All data associated with this study are present in the paper.

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